

CLAIMS

1. A liquid pharmaceutical composition in the form of an aqueous solution consisting essentially of an erythropoietin glycoprotein product having the *in vivo* biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells, a multipli-charged inorganic anion, a pharmaceutically acceptable buffer, said anion and said buffer being present in said solution in an amount to provide the solution with a pH of from 5.5 to about 7.0, said product being present in said solution in a sufficient amount to provide a therapeutically effective amount of said product when the solution is administered to a patient.
2. The composition of claim 1 wherein said solution is an isotonic solution.
3. The composition of claim 1 wherein the anion is an anion of a multipli-charged strong inorganic acid.
4. The composition of claim 3 wherein the anion is selected from the group consisting of sulfate, citrate or phosphate.
5. The composition of claim 4 wherein the anion is a sulfate anion.
6. The composition of claim 1 wherein the pH is 5.8 to 6.7
7. The composition of claim 6 wherein the pH is 6.0 to 6.5
8. The composition of claim 7 wherein the pH is about 6.2.
9. The composition of claim 1 wherein the buffer is selected form the group consisting of a phosphate or arginine/H₂SO₄/Na₂SO₄ buffers.

10. The composition of claim 9 wherein the buffer is a phosphate buffer in an amount of from 10 to 50 mmol per liter of said solution.

11. The composition of claim 1 wherein the product is a human erythropoietin.

12. The composition of claim 11 wherein the erythropoietin has the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:2.

13. The composition of claim 12 wherein the amino acid sequence of the erythropoietin is modified by the addition of from 1 to 6 glycosylation sites.

14. The composition of claim 14 wherein the sequence modification is selected from the group consisting of:

Asn³⁰Thr³²;
Asn⁵¹Thr⁵³;
Asn⁵⁷Thr⁵⁹;
Asn⁶⁹;
Asn⁶⁹Thr⁷¹;
Ser⁶⁸Asn⁶⁹Thr⁷¹;
Val⁸⁷Asn⁸⁸Thr⁹⁰;
Ser⁸⁷Asn⁸⁸Thr⁹⁰;
Ser⁸⁷Asn⁸⁸Gly⁸⁹Thr⁹⁰;
Ser⁸⁷Asn⁸⁸Thr⁹⁰Thr⁹²;
Ser⁸⁷Asn⁸⁸Thr⁹⁰Ala¹⁶²;
Asn⁶⁹Thr⁷¹Ser⁸⁷Asn⁸⁸Thr⁹⁰;
Asn³⁰Thr³²Val⁸⁷Asn⁸⁸Thr⁹⁰;
Asn⁸⁹Ile⁹⁰Thr⁹¹;
Ser⁸⁷Asn⁸⁹Ile⁹⁰Thr⁹¹;
Asn¹³⁶Thr¹³⁸;

Asn¹³⁸Thr¹⁴⁰;
Thr¹²⁵; and
Pro¹²⁴Thr¹²⁵.

15. The composition of claim 12, wherein the erythropoietin has the sequence of human erythropoietin modified by a rearrangement of at least one glycosylation site.

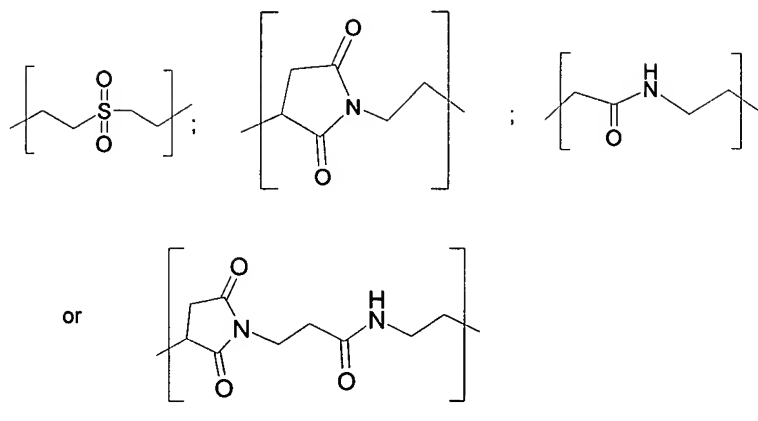
16. The composition of claim 15, wherein the rearrangement comprises deletion of any of the N-linked glycosylation sites in human erythropoietin sequence with the addition of an N-linked glycosylation site at position 88 of the sequence of human erythropoietin.

17. The composition of claim 16, wherein the sequence modification is selected from the group consisting of

Gln²⁴ Ser⁸⁷ Asn⁸⁸ Thr⁹⁰;
Gln³⁸ Ser⁸⁷ Asn⁸⁸ Thr⁹⁰; and
Gln⁸³ Ser⁸⁷ Asn⁸⁸ Thr⁹⁰.

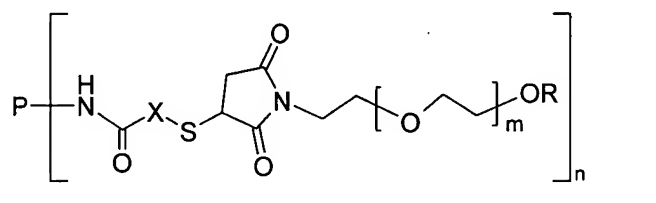
18. The composition of claim 1, wherein the glycoprotein product is a pegylated erythropoietin.

19. A liquid pharmaceutical composition in the form of an aqueous solution consisting essentially of a pegylated erythropoietin glycoprotein conjugate, said glycoprotein having at least one free amino group and having the sequence SEQ ID NO: 1 or SEQ ID NO: 2 or having said sequence modified by the addition of from 1 to 6 glycosylation sites; said glycoprotein being covalently linked to from one to three lower-alkoxy poly(ethylene glycol) groups with each poly(ethylene glycol) group being covalently linked to the glycoprotein via a linker of the formula -C(O)-X-S-Y- with the C(O) of the linker forming an amide bond with one of said amino groups; X is -(CH₂)_k- or -CH₂(O-CH₂-CH₂)_k-; k is from 1 to 10; Y is selected from



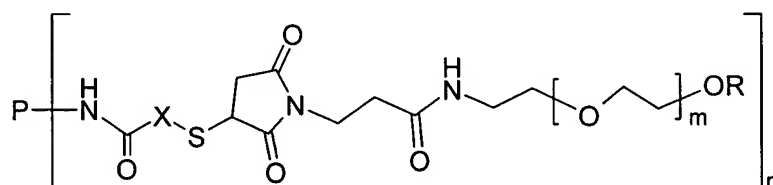
the average molecular weight of each poly(ethylene glycol) moiety being from about 20 kilodaltons to about 40 kilodaltons; and wherein the molecular weight of the pegylated erythropoietin glycoprotein is from about 51 kilodaltons to about 175 kilodaltons; said liquid composition being stable at room temperature.

20. The composition of claim 19 wherein said conjugate has the formula:



wherein n is an integer from 1 to 3; m is an integer from 450 to 900; R is lower alkyl; X is $-(\text{CH}_2)_k-$ or $-\text{CH}_2(\text{O}-\text{CH}_2-\text{CH}_2)_k-$; and P is the erythropoietin glycoprotein without the amino group or groups which form an amide linkage; and k is from 1-10.

21. The composition of claim 19 wherein the conjugate has the formula:



wherein n is an integer from 1 to 3; m is an integer from 450 to 900; R is lower alkyl; X is $-(CH_2)_k-$ or $-CH_2(O-CH_2-CH_2)_k-$; and P is the erythropoietin glycoprotein without the amino group or groups which form an amide linkage; and k is from 1-10.

22. A liquid pharmaceutical composition in the form of an aqueous solution consisting essentially of from 10 μ g to 10,000 μ g per ml of said solution of an erythropoietin glycoprotein product having the *in vivo* biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells, from 10 to 200 mmol per liter of said solution of a multipli-charged inorganic anion and from 10 to 50 mmol per liter of said solution of a pharmaceutically acceptable buffer, said anion and said buffer being present in said solution in an amount to provide the solution with a pH of from 5.5 to about 7.0; said liquid pharmaceutical composition being stable at room temperature.

23. The composition of claim 22 wherein said solution is an isotonic solution.

24. The composition of claim 22 wherein the anion is an anion of a multipli-charged strong inorganic acid.

25. The composition of claim 24 wherein the anion is selected from the group consisting of sulfate, citrate or phosphate.

26. The composition of claim 25 wherein the anion is a sulfate anion.

27. The composition of claim 26 wherein the pH is 5.8 to 6.7

28. The composition of claim 26 wherein the pH is 6.0 to 6.5

29. The composition of claim 26 wherein the pH is about 6.2.
30. The composition of claim 22 wherein the buffer is selected from the group consisting of phosphate or arginine/H₂SO₄/Na₂SO₄ buffers.
31. The composition of claim 30 wherein the buffer is a phosphate buffer in an amount of from 10 to 50 mmol per liter of said solution.
32. The composition of claim 22 wherein the product is a human erythropoietin.
33. The composition of claim 32 wherein the erythropoietin has the amino acid sequence SEQ ID NO:1 or SEQ ID NO:2.
34. The composition of claim 22 wherein said erythropoietin glycoprotein product has the sequence SEQ ID NO: 1 or SEQ ID NO: 2 that is modified by the addition of from 1 to 6 glycosylation sites.
35. The composition of claim 34 wherein the sequence modification is selected from the group consisting of:
- Asn³⁰Thr³²;
 - Asn⁵¹Thr⁵³;
 - Asn⁵⁷Thr⁵⁹;
 - Asn⁶⁹;
 - Asn⁶⁹Thr⁷¹;
 - Ser⁶⁸Asn⁶⁹Thr⁷¹;
 - Val⁸⁷Asn⁸⁸Thr⁹⁰;
 - Ser⁸⁷Asn⁸⁸Thr⁹⁰;
 - Ser⁸⁷Asn⁸⁸Gly⁸⁹Thr⁹⁰;
 - Ser⁸⁷Asn⁸⁸Thr⁹⁰Thr⁹²;

Ser⁸⁷Asn⁸⁸Thr⁹⁰Ala¹⁶²;
Asn⁶⁹Thr⁷¹Ser⁸⁷Asn⁸⁸Thr⁹⁰;
Asn³⁰Thr³²Val⁸⁷Asn⁸⁸Thr⁹⁰;
Asn⁸⁹Ile⁹⁰Thr⁹¹;
Ser⁸⁷Asn⁸⁹Ile⁹⁰Thr⁹¹;
Asn¹³⁶Thr¹³⁸;
Asn¹³⁸Thr¹⁴⁰;
Thr¹²⁵; and
Pro¹²⁴Thr¹²⁵.

36. The composition of claim 22 wherein said erythropoietin glycoprotein product has the sequence SEQ ID NO: 1 or SEQ ID NO: 2 that is modified by a rearrangement of at least one glycosylation site.

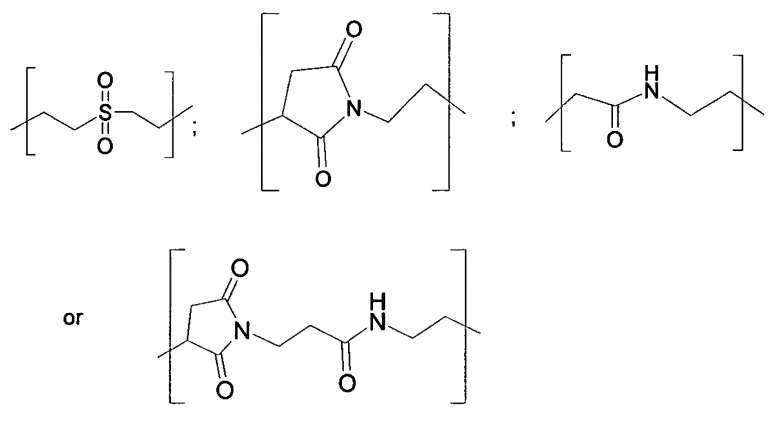
37. The composition of claim 36, wherein the rearrangement comprises deletion of any of the N-linked glycosylation sites in human erythropoietin with the addition of an N-linked glycosylation site at position 88 of the sequence of human erythropoietin.

38. The composition of claim 37, wherein the sequence modification is selected from the group consisting of:

Gln²⁴Ser⁸⁷Asn⁸⁸Thr⁹⁰;
Gln³⁸Ser⁸⁷Asn⁸⁸Thr⁹⁰; and
Gln⁸³Ser⁸⁷Asn⁸⁸Thr⁹⁰.

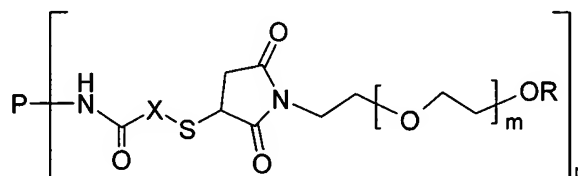
39. The composition of claim 22, wherein said glycoprotein product is a pegylated erythropoietin glycoprotein product.

40. The composition of claim 39, wherein the pegylated erythropoietin glycoprotein product is a conjugate of an erythropoietin glycoprotein having at least one free amino group, said glycoprotein having the sequence SEQ ID NO: 1 or SEQ ID NO: 2 or having said sequence modified by the addition of from 1 to 6 glycosylation sites; said glycoprotein being covalently linked to from one to three lower-alkoxy poly(ethylene glycol) groups with each poly(ethylene glycol) group being covalently linked to the glycoprotein *via* a linker of the formula -C(O)-X-S-Y- with the C(O) of the linker forming an amide bond with one of said amino groups, X is -(CH₂)_k- or -CH₂(O-CH₂-CH₂)_k-; and k is from 1 to 10; Y is selected from



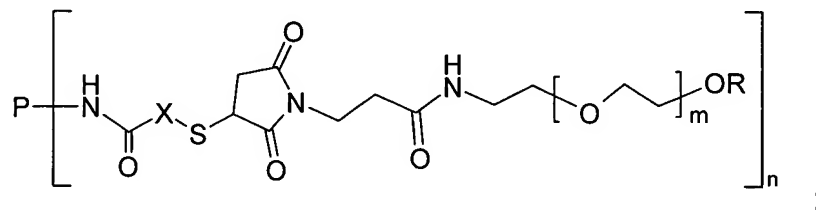
the average molecular weight of each poly(ethylene glycol) moiety being from about 20 kilodaltons to about 40 kilodaltons; and the molecular weight of the conjugate being from about 51 kilodaltons to about 175 kilodaltons.

41. The composition of claim 40 wherein said conjugate has the formula:



wherein n is an integer from 1 to 3; m is an integer from 450 to 900; R is lower alkyl; X is $-(CH_2)_k-$ or $-CH_2(O-CH_2-CH_2)_k-$, and P is the erythropoietin glycoprotein minus the amino group or groups which form an amide linkage with X and k is from 1 to 10.

42. The composition of claim 41 wherein said conjugate has the formula:



n is an integer from 1 to 3; m is an integer from 450 to 900; R is lower alkyl; X is $-(CH_2)_k-$ or $-CH_2(O-CH_2-CH_2)_k-$, and P is the erythropoietin glycoprotein minus the amino group or groups which form an amide linkage; and k is from 1 to 10.

43. The composition of claim 22 wherein said solution contains 10 µg to 10000 µg erythropoietin protein per ml of solution, from 10 to 200 mmol/liter of solution of a sulfate as the multipli-charged inorganic anion, and 10 to 50 mmol/liter of solution of a phosphate as the pharmaceutically acceptable buffer, said solution having a pH of from about 6.0 to about 6.5.

44. The composition of claim 43 further containing up to 20 mM of methionine and 1 - 5 % of a polyol (w/v).

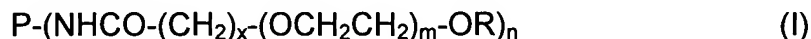
45. The composition of claim 44 consisting essentially of 10 µg to 10000 µg erythropoietin protein per ml of solution, 40 mmol/liter of solution of the sulfate, 10 mmol/liter of said solution of the phosphate, 10 mM methionine, said composition having a pH of about 6.2, and wherein the polyol is mannitol which is present in the solution at 3% (w/v).

46. The composition of claim 22 wherein the solution contains 10 µg to 10000 µg erythropoietin protein per ml of solution, the buffer is phosphate which is present at 10 to 50 mmol/liter of solution, said solution further containing NaCl which is present at 10 to 100 mmol/liter of solution and having a pH of from about 6.0 to about 7.0.

47. The composition of claim 46 wherein the NaCl is present at 100 mmol/liter of solution, the phosphate is present at 10 mmol/l, said solution further containing 10 mM methionine and having a pH of about 7.0.

48. The composition of claim 22 wherein the amount of erythropoietin is 50, 100, 400, 800 or 2,500 µg/ml of solution.

49. A liquid pharmaceutical composition in the form of an aqueous solution consisting essentially of a therapeutically effective amount of a pegylated erythropoietin glycoprotein product of formula



wherein

P is an erythropoietin glycoprotein having the sequence SEQ ID NO: 1, SEQ ID NO: 2, or either of these sequences modified by the addition of from 1 to 6 glycosylation sites or by rearrangement of at least one glycosylation site, minus the amino group of said glycoprotein which forms an amide linkage;

R is lower alkyl;

x is 2 or 3;

m is from about 450 to about 900;

n is from 1 to 3; and

wherein the values of n and m are such that the molecular weight of the conjugate minus the erythropoietin glycoprotein is from 20 kilodaltons to 100 kilodaltons; and

a multipli-charged inorganic anion and a pharmaceutically acceptable buffer, said anion and said buffer being present in said solution in an amount such that the pH of the solution is from about 5.5 to about 7.0.

50. The composition of claim 49 wherein x is 2, m is 650 to 750, n is 1, and R is methyl.

51. The liquid pharmaceutical composition of claim 49 wherein the pegylated erythropoietin glycoprotein product is present in an amount of from about 10 µg to about 10,000 µg per ml of said liquid composition, the multipli-charged inorganic anion is present in an amount of from 10 to 200 mmol per liter of said liquid composition, and the pharmaceutically acceptable buffer is present in an amount of from about 10 to about 50 mmol per liter of said liquid composition, said anion and said buffer being present in said liquid composition in an amount to provide a pH of from about 5.5 to about 7.0.

52. The composition of claim 51 wherein x is 2, m is 650 to 750, n is 1, and R is methyl.

53. The liquid pharmaceutical composition of claim 50 wherein the pegylated erythropoietin glycoprotein is present in an amount of about 100.0 µg/mL of solution, the multipli-charged inorganic anion is sodium sulphate which is present in an amount of about 5.68 mg/mL, the pharmaceutically acceptable buffer is sodium phosphate which is present in an amount of about 1.38 mg/mL, and wherein the pH of the solution is about 6.2 ± 0.2 .

54. The liquid pharmaceutical composition of claim 53 further comprising methionine in an amount of about 1.49 mg/mL, mannitol in an amount of about 30.0 mg/mL and poloxamers type 188 in an amount of 0.1 mg/mL.

55. The liquid pharmaceutical composition of claim 50 wherein the pegylated erythropoietin glycoprotein is present in an amount of about 400 µg/mL of solution, the multipli-charged inorganic anion is sodium sulphate which is present in an amount of about 5.68 mg/mL, the pharmaceutically acceptable buffer is sodium phosphate which is present in an amount of about 1.38 mg/mL, and wherein the pH of the solution is about 6.2 ± 0.2 .

56. The liquid pharmaceutical composition of claim 55 further containing methionine in an amount of about 1.49 mg/mL, mannitol in an amount of about 30.0 mg/mL and poloxamers type 188 in an amount of 0.1 mg/mL.

57. The liquid pharmaceutical composition of claim 50 wherein the pegylated erythropoietin glycoprotein is present in an amount of about 800.0 µg/mL of solution, the multipli-charged inorganic anion is sodium sulphate which is present in an amount of about 5.68 mg/mL, the pharmaceutically acceptable buffer is sodium phosphate which is present in an amount of about 1.38 mg/mL, and wherein the pH of the solution is about 6.2 ± 0.2 .

58. The liquid pharmaceutical composition of claim 57 further containing methionine in an amount of about 1.49 mg/mL, mannitol in an amount of about 30.0 mg/mL and poloxamers type 188 in an amount of 0.1 mg.

59. The composition of claim 22 wherein the erythropoietin is present at about 25 to about 2,500 µg/ml, the buffer is sodium or potassium phosphate which is present in an amount of about 10 mM, said composition further containing NaCl which is present in an amount of about 100 mM-and having a pH of about 7.0.